IL-18 Is Induced and IL-18 Receptor α Plays a Critical Role in the Pathogenesis of Cigarette Smoke-Induced Pulmonary Emphysema and Inflammation

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IL-18 Is Induced and IL-18 Receptor α Plays a Critical Role in the Pathogenesis of Cigarette Smoke-Induced Pulmonary Emphysema and Inflammation

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Diseases that are generated by exaggerated Th1/Tc1 tissue responses are frequently characterized by macrophage- and/or neutrophil-rich inflammation and tissue atrophy and destruction. This can be readily appreciated in the joint erosions in rheumatoid arthritis (1), atrophy, and fistulization in inflammatory bowel disease (2), histologic necrosis in mycobacterial infections and granulomatous diseases (3), pancreatic destruction in diabetes (4), and neurodegeneration in multiple sclerosis (5, 6). This is also seen in studies of human pulmonary emphysema that demonstrated that alveolar septal rupture and enhanced production of type I cytokines and type I cytokine-stimulated genes are juxtaposed (7–11), and studies from our laboratory that demonstrated that IFN-γ plays an important role in the pathogenesis of emphysema in murine modeling systems (12–14). Interestingly, chronic obstructive pulmonary disease (COPD) (the disease spectrum that includes pulmonary emphysema) may not be mediated solely by Th1 responses because tissue eosinophilia and Th2 cytokines are well documented in tissues and sputum from these patients and individuals with features of COPD and asthma (a Th2-dominated disease) are frequently encountered (15–20). Surprisingly, the contributions of Th1 cytokines other than IFN-γ and the mechanisms by which type I and type II immune responses can both be induced in COPD have not been addressed.

IL-18 was described in 1995 as IFN-γ-inducing factor and shown to be a member of the IL-1 cytokine superfamily (21–23). It is produced as a 23- to 24-kDa inactive propeptide, is activated by caspase-1 to an 18-kDa active moiety, and mediates its effects by binding to a heterodimeric receptor made up of a ligand binding subunit and a signaling subunit (reviewed in Refs. 21–23). It is now appreciated to be an important regulator of innate and adaptive immunity. It has impressive effects on type I immune responses where it induces Th1/Tc1 lineage differentiation and T and NK cell maturation, stimulates IFN-γ production, and regulates macrophage and neutrophil accumulation and function and cellular apoptosis (21–23). Interestingly, in the presence of IL-4 or IL-2, it can also contribute to Th2 responses by stimulating IgE production and Th2 cell differentiation (21–23). However, despite its important contributions to type I and II immune responses, the role of IL-18 in the pathogenesis of pulmonary emphysema has not been addressed.

We hypothesized that IL-18 is induced during and plays a key role in the pathogenesis of pulmonary emphysema. To test this hypothesis, we characterized the effects of cigarette smoke (CS) on IL-18 and the caspases that activate it and characterized the in inflammatory and destructive effects of CS in wild-type (WT) mice...
FIGURE 1. Regulation of IL-18 by CS. WT mice were exposed to RA (nonsmoking, NS) or CS for 2 wk to 2 mo. Whole lung RNA was extracted and the levels of IL-18 mRNA were evaluated via real-time RT-PCR (A). The levels of IL-18 protein in 5× concentrated BAL fluids (B) and 100 μg of whole lung protein lysates (C) were evaluated by ELISA, and the propeptide and activated form of IL-18 was also interrogated using Western blot analysis (D). The location of IL-18 was also evaluated using IHC evaluations (E). The values in A–C represent the mean ± SEM of evaluations in a minimum of five mice. The evaluations in D and E are representative of at least four similar experiments (*, p < 0.05; **, p < 0.01). The arrows in E highlight positively staining macrophages.

and mice with null mutations of IL-18Ra. These studies demonstrate that IL-18, caspase-1 and caspase 11 are induced by CS, that signaling via IL-18Ra is a critical event in the pathogenesis of CS-induced inflammation and emphysema and that CS induces epithelial apoptosis, stimulates proteases and chemokines and activates a variety of caspsases via IL-18R-dependent mechanisms. We also demonstrate that IL-18 is present in exaggerated quantities in the lungs from smokers and patients with COPD and that elevated levels of IL-18 can be detected in the serum from patients with COPD.

Materials and Methods

CS exposure

C57BL/6 WT mice and IL-18Ra−/− mice were purchased from The Jackson Laboratory. Starting at 10 wk of age, they were exposed twice a day, 5 days a week, to room air (RA) or the smoke from two nonfiltered standard research cigarettes (2R4, University of Kentucky) (CS) using the smoking apparatus described by Hautamaki et al. (24). At the 0.5- to 3-mo time points, bronchoalveolar lavage fluid (BAL) and TUNEL evaluations were undertaken as described below. After 6 mo, the mice were anesthetized and sacrificed, and the trachea was cannulated. After ligation of the right main bronchus, the left lung was inflated with 0.5% low temperature-melting agarose in 10% PBS-buffered formalin at a constant pressure of 25 cm. This allowed for homogenous expansion of lung parenchyma as described by Halbower et al. (25). The lungs were then fixed in 10% PBS-buffered formalin, fixed overnight in 10% formalin, embedded in paraffin, sectioned, and stained. H&E stains were performed in the Research Histology Laboratory of the Department of Pathology at Yale University School of Medicine.

mRNA analysis

mRNA levels were assessed using real-time RT-PCR as previously described by our laboratory (12, 13, 26). In these assays, gene-specific primers were used to amplify selected regions of each target moiety. The primers for targeted genes have been described in our publications or are detailed below: caspase-1, forward, 5′-acc ctc aag ttt tgc cct tt-3′, and reverse, 5′-tct ggt tcc tcc att tcc ag-3′; and IL-18, forward, 5′-gac aag cgt tgg gtt ttt gt-3′, and reverse, 5′-gac aag cgt tgg gtt ttt gt-3′.

Immunohistochemistry (IHC)

IHC was undertaken as described previously (12, 13, 26). Primary Abs against murine IL-18 (Santa Cruz Biotechnology), cathepsin-S (Santa Cruz Biotechnology), or activated caspase-3 (Promega) were used.

Histologic analysis

Animals were anesthetized, a median sternotomy was performed and right heart perfusion was accomplished with calcium- and magnesium-free PBS to clear the pulmonary intravascular space. The lungs were then fixed to pressure (25 cm) with neutral-buffered 10% formalin, fixed overnight in 10% formalin, embedded in paraffin, sectioned, and stained. H&E stains were performed in the Research Histology Laboratory of the Department of Pathology at Yale University School of Medicine.
**FIGURE 2.** Regulation of caspases 1 and 11 by CS. Real-time RT-PCR evaluations of whole lung RNA and Western blot evaluations were used to quantify the levels of caspase-1 mRNA (A), size the caspases 1 and 11 enzymes (B), and quantify the levels of caspase-11 mRNA (C) in lungs from mice exposed to CS or RA for 2 mo. In the Western blot evaluations, the propeptide (pro) and activated (active) forms of caspase-1 are noted. The values in A and C represent the mean ± SEM of evaluations in a minimum of five mice. The evaluations in B are representative of at least four similar experiments (*, p < 0.05; **, p < 0.01).

**Chemokine measurements**

The levels of selected chemokines in BAL were evaluated by ELISA using commercial assays (R&D Systems) as described by the manufacturer.

**Lung volume, morphometric, and compliance assessment**

Lung volume, alveolar size, and lung compliance were assessed via volume displacement and morphometric chord length assessments as described previously (12, 13, 26).

**TUNEL evaluations**

End labeling of exposed 3'-OH ends of DNA fragments in paraffin-embedded tissue was undertaken with the TUNEL in situ cell death detection kit AP (Roche Diagnostics) using the instructions provided by the manufacturer. Staining specificity was assessed by comparing the signal that was seen when terminal transferase was included and excluded from the reaction. After staining, a minimum of 20 fields of alveoli were randomly chosen, and 500 nuclei were counted per lung. The labeled cells were expressed as a percentage of total nuclei.

**Murine caspase and immunoblot evaluations**

Whole lung lysates were prepared, and Western blot evaluations were undertaken as previously described by our laboratory (12, 13, 26). Abs that selectively reacted to IL-18, caspase-3, caspase-8, cathepsin-S, cathepsin-B, and β-tubulin were purchased from Santa Cruz Biotechnology. Commercial sources were also used for the Abs against caspase-1 (Upstate Biotechnology), caspase-11 (Abcam), and caspase-12 (Cell Signaling Technology).

**Immunohistochemical evaluation of human lung tissues**

We identified 44 patients who had undergone lung resection at Yale between 1995 and 1999 who had adequate clinical data and tissue blocks available for evaluation. Clinical data were extracted using a standardized chart abstraction form by an investigator blinded to the results of the tissue staining. Pulmonary function tests performed within the 1 year before surgery were obtained from the chart. Patients were divided into groups based on smoking status and the presence or absence of COPD. The smoking status categories included current smokers (any use within 1 year before surgery), former smokers (last use >1 year before surgery), and never-smokers. The never-smokers were generally undergoing resection for a metastatic neoplasm. The current smokers and former smokers were generally undergoing resection for non-small cell lung cancer. Patients were defined as having COPD if they had 1) documentation of COPD according to physician notes in the medical record, 2) an active smoking status with a forced expiratory volume in 1 s/forced vital capacity ratio <70% without another explanation, and 3) an active smoking status without any other lung disease with a carbon monoxide diffusing capacity (DL_{CO}) <80% predicted and computerized tomography scan evidence of emphysema. Classification using guidelines from the Global Initiative for Chronic Obstructive Lung Disease (GOLD) was also undertaken. These patients are described in Table I. This study was approved by the Yale Human Investigation Committee.

Blocks of lung away from the neoplasm were selected. Abs to cathepsin-S (SC-6503, Santa Cruz Biotechnology; clone 1E3, Krka) (27) and cathepsin-B (SC-6493, Santa Cruz Biotechnology; clone 3E1, Krka) (28) were used with heat-induced Ag retrieval in 10 mM citrate (pH 6.0). Ab to IL-18 (clone 8; Dr. D.-Y. Yoon, Laboratory of Cell Biology, Korean Research Institute of Bioscience and Biotechnology, Daejeon, Korea) (29) was used at a titer of 1/1000 overnight at 4 degrees using Ag retrieval with Vector (pH 6.0) Ag retrieval solution (Vector Laboratories). Staining was developed with biotinylated anti-mouse or anti-goat Ab (1/100; Vector Laboratories), streptavidin-alkaline phosphatase (Vector Laboratories), and Vector Red, according to the manufacturer’s instructions. Specificity was determined by lack of staining by negative control antisera, identical results with both mouse and goat Abs (for cathepsins S and B), similarity to previously published results, specificity by Western blot and peptide inhibition by peptide immunogen (for cathepsins S and B). Sections were scored on a 0–4 scale (0 = no staining, 4 = strong diffuse staining) based on a global assessment of staining (intensity per cell and number of cells stained) by a pathologist blinded to the clinical information associated with each slide. A minimum of 10 nonoverlapping fields were evaluated for each patient.

**FIGURE 3.** Role of IL-18Rα in CS-induced emphysema. WT and IL-18Rα−/− mice were exposed to RA (nonsmoking, NS) or CS for 6 mo. Histologic analysis (A) and morphometric assessments of chord length (B) were then undertaken. The values in B represent the mean ± SEM of evaluations of a minimum of eight mice. The evaluations in A are representative of at least four similar experiments (**, p < 0.01).
Informed consent was obtained on all study participants. A total of 29 patients with COPD and 78 age-matched controls seen in the pulmonary and primary care clinics of the West Haven Veterans Administration Medical Center between January 2005 and April 2006 was enrolled. The diagnosis of COPD, the demographics of the patients, and their comorbid diseases were obtained from a standardized chart review. Smoking histories

Quantification of serum IL-18

Informed consent was obtained on all study participants. A total of 29 patients with COPD and 78 age-matched controls seen in the pulmonary and primary care clinics of the West Haven Veterans Administration Medical Center between January 2005 and April 2006 was enrolled. The diagnosis of COPD, the demographics of the patients, and their comorbid diseases were obtained from a standardized chart review. Smoking histories

FIGURE 4. Role of IL-18Rα in CS-induced inflammation. WT and IL-18Rα−/− mice were exposed to CS or RA (CS −) for the noted intervals. Total BAL cell recovery (A), macrophage recovery (1 mo) (B), and neutrophil recovery (1 mo) (C) are illustrated. D, WT mice were exposed to RA or CS for 1 mo. During this interval the mice were treated with Ab against IL-18Rα or an appropriate control. Total BAL cell recovery is illustrated. The values in A–D represent the mean ± SEM of evaluations of a minimum of eight mice (*, p < 0.05; **, p < 0.01).

FIGURE 5. Role of IL-18Rα in CS-induced apoptosis. WT mice and IL-18Rα−/− mice were exposed to RA (nonsmoking, NS; CS −) or CS for the noted intervals. TUNEL (A and B), IHC for activated caspase-3 (C), and Western blot caspase (D) evaluations were undertaken. In the Western blot evaluations, the propeptide (Pro) and activated (cleaved) forms of the caspases are noted. The values in B represent the mean ± SEM of evaluations in a minimum of five mice. The evaluations in A, C, and D are representative of at least four similar experiments (*, p < 0.05; **, p < 0.01). The arrows in A and C highlight TUNEL staining and activated caspase-3 staining alveolar type II cells, respectively.
were obtained from the chart reviews and standardized interviews performed by one investigator (A. Gallo) who was blinded to the results of the patient’s IL-18 evaluations. Subjects with a history of asthma, other lung diseases, lung resection, HIV, or hepatitis C were excluded. Subjects that were immunocompromised or taking oral corticosteroids were also excluded. Once the chart review and interview were completed, the patients were placed into two groups: patients without COPD and patients with COPD (a physician diagnosis and a forced expiratory volume in 1 s/forced vital capacity ratio < 70%) (see Table III). In all cases, peripheral blood samples were collected in non-coated tubes, the serum was separated by centrifugation, and the specimens were stored at −80 °C until used. The levels of IL-18 were determined by ELISA using commercial kits according to the manufacturer’s instructions (R&D Systems). This research was approved by the Yale University and West Haven Veterans Administration Institutional Review Boards. There was no overlap between these patients and the patients whose lung resection specimens were evaluated immunohistochemically.

Statistics

Normally distributed data are expressed as mean ± SEM and were assessed for significance by Student’s t test or ANOVA as appropriate. Data that were not normally distributed are expressed as media with interquartile ranges and were assessed for significance using the Mann-Whitney U test or the Kruskal-Wallis test with Dunn’s posttest for multiple comparisons as appropriate. Statistical analysis was performed using Stata version 9.0 (StataCorp), Deltagraph (RockWare), and Prism version 4 (GraphPad). Statistical significance was defined at a level of p ≤ 0.05.

Results

CS regulation of IL-18

To address the role of IL-18 in the pathogenesis of CS-induced pulmonary responses, studies were undertaken to determine whether CS regulated the expression and/or production of this cytokine in the murine lung. IL-18 mRNA and protein were readily appreciated in lungs from mice breathing RA (Fig. 1, A and B). Interestingly, the levels of IL-18 mRNA and protein were increased significantly after CS exposure (Fig. 1, A and B). The increases in mRNA were readily apparent after as little as 2 wk of CS exposure, were readily appreciated after 1–3 mo of CS exposure, and persisted throughout the study interval (Fig. 1A and data not shown). In all cases, they were associated with comparable increases in BAL and lysate IL-18 protein when assessed by ELISA (Fig. 1, B and C, and data not shown), immunoblot (Fig. 1D), or IHC evaluations (Fig. 1E). The immunoblots also demonstrated that this stimulation was associated with modest increases in the levels of the IL-18 propeptide and more impressive increases in the levels of the mature IL-18 cytokine (Fig. 1D). The IHC demonstrated that the major site of IL-18 protein localization was in alveolar macrophages (Fig. 1E).

CS regulation of caspase-1 and caspase-11

Studies were next undertaken to determine whether CS regulated the production and/or activation of the IL-18 activator, caspase-1. Caspase-1 mRNA and protein were readily detected in lungs from mice breathing RA (Fig. 2, A and B). At the protein level, the majority of this pool was made up of inactive caspase-1 precursor moieties (Fig. 2B). In contrast to mice breathing RA, increased levels of caspase-1 mRNA and activated caspase-1 protein were noted in lungs from mice exposed to CS (Fig. 2, A and B). This increase in activated caspase-1 could be detected after as little as 2 wk and persisted with
longer periods (2 mo) of CS exposure (data not shown). It was also associated with significantly increased levels of activated caspase-11 and the mRNA encoding this known caspase-1 activator (Fig. 2, B and C). Thus, CS increases the levels of active caspase-1 and stimulates caspase-11 while simultaneously increasing the production and activation of IL-18 in the murine lung.

Role of the IL-18R in CS-induced emphysema

To determine whether IL-18 signaling contributes to the pathogenesis of CS-induced emphysema, WT and IL-18R α−/− mice were exposed to CS for 6 mo and the alveoli in lungs from these animals were compared. In accord with studies from our laboratory and others (12, 24), 6 mo of cigarette exposure caused modest alveolar destruction, which could be appreciated in histologic sections (Fig. 3A) and morphometric evaluations (Fig. 3B). This alveolar simplification was significantly ameliorated in IL-18R α−/− animals (Fig. 3). Overall, a null mutation of IL-18R α caused a 51.5% decrease in the CS-induced increase in alveolar chord length (p < 0.01). Thus, IL-18R α plays a critical role in the pathogenesis of CS-induced pulmonary emphysema.

Role of IL-18R α in CS-induced inflammation

The current concept of emphysema disease pathogenesis suggests that inflammation contributes, in a causal way, to the genesis of pulmonary emphysema. Thus, studies were undertaken to determine whether genetic alterations in the IL-18 receptor altered CS-induced inflammation in a manner that is analogous to their effects on alveolar remodeling. When compared with mice breathing RA, BAL from mice exposed for 1–3 mo to CS manifest significant increases in total cell recovery and increases in macrophage, and to a lesser extent, neutrophil recovery (Fig. 4, A–C, and data not shown). These BAL alterations were associated with patchy increases in tissue cellularity where increased numbers of macrophages were readily appreciated (data not shown). In all cases, these responses were IL-18R α dependent because the BAL and tissue cellular responses were both significantly decreased in IL-18R α−/− animals and WT mice treated with neutralizing Abs against IL-18R α (Fig. 4). Thus, CS induces pulmonary inflammation via an IL-18R α-dependent mechanism.

Role of IL-18R in CS-induced apoptosis

Studies were next undertaken to determine whether CS induced tissue apoptosis and IL-18 contributed to the pathogenesis of this cell death response. This was done by comparing the levels of DNA injury and cell death (using TUNEL evaluations) and the levels of activated caspases 3 and 8 in lungs from WT and IL-18R α−/− mice exposed to RA or CS. Cells that were TUNEL positive were not readily appreciated and only low levels of activated caspases −3 and −8 were appreciated in lungs from WT and
IL-18R/-null mice breathing RA (Fig. 5). In contrast, apoptosis of alveolar type II epithelial cells and occasional airway epithelial cells was readily appreciated and increased levels of activated caspase-3 and caspase-8 were detected in lungs from WT mice exposed to CS for 1–3 mo (Fig. 5 and data not shown). Similarly, the caspase-12 proenzyme was readily appreciated in lungs from mice breathing RA, while increased levels of this proenzyme and activated caspase-12 were seen after CS exposure (Fig. 5). Importantly, each of these responses was significantly ameliorated in mice with null mutations of IL-18R (Fig. 5). Thus, IL-18R signaling contributes, in a significant fashion, to the pathogenesis of CS-induced cell injury and death and caspase activation in the murine lung.

Role of IL-18 signaling in CS-induced protease alterations

Studies were next undertaken to determine whether IL-18Rα contributed to CS-induced alterations in proteases in the murine lung. This was done by comparing the expression of matrix metalloproteinase (MMP)-12 and cathepsins -S and -B in lungs from RA and CS-exposed IL-18Rα−/− and IL-18Rα−/− mice. Comparable levels of mRNA encoding these proteases were appreciated in lungs from mice with WT and null IL-18Rα loci breathing RA (Fig. 6, A–C). In addition, the levels of mRNA encoding these moieties increased after 1–3 mo of CS exposure in WT mice (Fig. 6, A–C). In contrast, comparable increases in the levels of mRNA encoding these moieties were not appreciated in lungs from IL-18Rα-null animals exposed to CS (Fig. 6, A–C). In all cases, alterations in
mRNA were associated with comparable alterations in protease protein (Fig. 6D and data not shown). IHC also demonstrated that the cathepsin alterations were largely localized to alveolar macrophages (Fig. 6E and data not shown). Thus, IL-18R signaling plays an important role in the pathogenesis of CS-induced protease alterations in macrophages in the murine lung.

Role of IL-18R in CS-induced chemokine responses

Previous studies from our laboratory and others have demonstrated that CS induces chemokine responses that play an important role in the pathogenesis of pulmonary emphysema (12, 30). Thus, the role of IL-18R signaling was evaluated. In accord with the prior studies (12, 30), CS induced IL-18 and cathepsin expression in lungs from current smokers (cathepsin-S, cathepsin-B) and data not shown). Quantitation showed a strong overall correlation with smoking status (cathepsin-S, cathepsin-B, p < 0.001, Kruskal-Wallis test) with higher levels of cathepsin-S and cathepsin-B in current vs never-smokers (cathepsin-S, cathepsin-B, p < 0.001, cathepsin-B p < 0.05 using Dunn’s test and in current vs former smokers (cathepsin-S, cathepsin-B, p < 0.01, using Dunn’s test) (Fig. 8, B and C).

One theoretical concern is that the presence of tumor could have influenced the expression of IL-18 and the cathepsins. Due to their small numbers, it was not possible to analyze only patients without lung malignancies. Thus, the analysis was restricted to the patients in the current and former smoking groups with lung cancer. Despite the presence of lung cancer in all patients, we still noted significantly higher levels of IL-18 and the cathepsins in tissues from current smokers than from never-smokers (p < 0.01, cathepsin-B, p < 0.05 using Dunn’s test). This indicates that the increased levels of IL-18 and cathepsins in tissues from current smokers are independent of the presence of lung cancer.

Evaluations were also undertaken to determine whether the levels of IL-18 and the cathepsins were influenced by COPD. Once again, statistically significantly elevated levels of IL-18 and the cathepsins in tissues from current smokers were independent of the presence of lung cancer.

Table I. Characteristics of patients undergoing lung resection according to smoking status

<table>
<thead>
<tr>
<th>Smoking Status</th>
<th>n</th>
<th>Age in years, median</th>
<th>Male sex N (%)</th>
<th>Race N (%)</th>
<th>Pack-years of smoking, median</th>
<th>Comorbidities</th>
<th>COPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current Smokers</td>
<td>18</td>
<td>59 (52–62)</td>
<td>7 (39)</td>
<td>Caucasian 17 (94)</td>
<td>53 (40–80)</td>
<td>13 (72%)</td>
<td>14 (78%)</td>
</tr>
<tr>
<td>Former Smokers</td>
<td>19</td>
<td>68 (56–76)</td>
<td>8 (42)</td>
<td>African American 16 (84)</td>
<td>45 (20–60)</td>
<td>12 (63%)</td>
<td>9 (47%)</td>
</tr>
<tr>
<td>Never Smokers</td>
<td>7</td>
<td>51 (31–65)</td>
<td>3 (43)</td>
<td>Hispanic 5 (71)</td>
<td>0</td>
<td>2 (29%)</td>
<td>0</td>
</tr>
</tbody>
</table>

* Value of p for comparison between all three groups, Kruskal-Wallis analysis. Medians are given with interquartile range.

Table II. Characteristics of patients undergoing lung resection according to COPD status

<table>
<thead>
<tr>
<th>COPD</th>
<th>n</th>
<th>Age in years, median</th>
<th>Race/ethnicity</th>
<th>Pack-years of smoking, median</th>
<th>GOLD stage</th>
<th>Lung malignancy</th>
<th>IL-18 score, median</th>
<th>Cathepsin-s score, median</th>
<th>Cathepsin-B score, median</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPD</td>
<td>23</td>
<td>60 (56–69)</td>
<td>Caucasian 19 (83%)</td>
<td>60 (40–80)</td>
<td>0</td>
<td>18 (78%)</td>
<td>3 (1–3)</td>
<td>2 (1–3)</td>
<td>2.5 (1–3)</td>
</tr>
<tr>
<td>No COPD</td>
<td>21</td>
<td>62 (50–68)</td>
<td>African American 4 (17%)</td>
<td>20 (20–50)</td>
<td>5</td>
<td>11 (52%)</td>
<td>1 (0–1)</td>
<td>1 (0–2)</td>
<td>1 (0–1.5)</td>
</tr>
</tbody>
</table>

* Value of p for comparison of both groups.
Table III. Characteristics of control and COPD serum donors

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 78)</th>
<th>COPD (n = 29)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>71 (60–76)</td>
<td>73 (66–78)</td>
<td>0.10</td>
</tr>
<tr>
<td>Male sex N (%)</td>
<td>74 (95)</td>
<td>27 (100)</td>
<td>0.19</td>
</tr>
<tr>
<td>Race N (%)</td>
<td>72 (92)</td>
<td>29 (100)</td>
<td>0.19</td>
</tr>
<tr>
<td>Caucasian</td>
<td>4 (5)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>2 (3)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Body mass index</td>
<td>29 (25–33)*</td>
<td>28 (24–33)**</td>
<td>0.48</td>
</tr>
<tr>
<td>Smoking history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>7 (9)</td>
<td>7 (24)</td>
<td>0.039</td>
</tr>
<tr>
<td>Former smoker</td>
<td>49 (63)</td>
<td>22 (76)</td>
<td>0.25</td>
</tr>
<tr>
<td>Never smoker</td>
<td>22 (28)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pack-year history</td>
<td>20 (0–40)</td>
<td>60 (45–90)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>21 (27)</td>
<td>11 (38)</td>
<td>0.27</td>
</tr>
<tr>
<td>Hypertension</td>
<td>40 (51)</td>
<td>10 (35)</td>
<td>0.13</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>17 (22)</td>
<td>4 (14)</td>
<td>0.42</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>19 (24)</td>
<td>8 (28)</td>
<td>0.80</td>
</tr>
<tr>
<td>Gastroesophageal reflux</td>
<td>7 (9)</td>
<td>4 (14)</td>
<td>0.49</td>
</tr>
</tbody>
</table>

* Data expressed as median (interquartile range) or N (%).
* p Value of p for comparison between control and COPD subjects.

Data missing on *, five subjects; **, one subject.

Discussion

To further define the cellular and molecular events involved in the pathogenesis of CS-induced inflammation and emphysema, we used in vivo approaches to characterize the effects of CS on IL-18 production and activation and defined the role of IL-18R signaling in the pathogenesis of these responses. These studies demonstrate that CS is a potent stimulator of IL-18 in the murine lung and that this stimulation is associated with IL-18 activation and the induction of the caspase-11-caspase-1 cascade that activates the IL-18 propeptide. They also demonstrate that CS-induced inflammation and emphysema are mediated via mechanisms that involve IL-18Rα and that CS-induction of apoptosis and stimulation of caspases (3, 8, and 12), proteases (MMP-12, cathepsin-S, and cathepsin-B) and chemokines (MCP-1, MCP-3, and MIP-1α) are mediated by IL-18Rα-dependent pathways. Lastly, human disease relevance is also provided by demonstrating that the levels of immunoreactive IL-18 and its downstream targets, cathepsin-S and cathepsin-B, are increased in macrophages in lungs from smokers and patients with COPD and that the levels of circulating IL-18 are increased in the serum of patients with COPD. When viewed in combination, these studies highlight the impressive activation of the IL-18-IL-18R pathway in response to CS in mice and man and demonstrate that IL-18R signaling plays a critical role in the pathogenesis of CS-induced inflammation and remodeling.

IL-18 is an inactive propeptide that is cleaved by caspase-1 to an active 18-kDa ligand that binds a heterodimeric receptor that contains a ligand binding α subunit and a signal transducing β subunit (21, 22). Our studies demonstrate that the IL-18 propeptide and mature peptide is induced by CS. They also demonstrate that these inductive events are associated with increased levels of caspase-1 and the caspase-1 stimulator, caspase-11 (32). Interestingly, CS induction of caspase-11 was mediated via an IL-18Rα-dependent pathway, whereas the stimulation of caspase-1 was not (M.-J. Kang and J. A. Elias, unpublished observation). These observations suggest that caspase-1 and caspase-11 play important roles in the activation of IL-18 in the CS-exposed lung. They also highlight a potential positive feedback loop in which IL-18 induces caspase-11, which, in turn, drives caspase-1 to further increase the levels of active IL-18. However, the ultimate importance of this pathway will need validation because, in addition to caspase-1, proteinase 3 and cathepsin-B can contribute to IL-18 activation (21, 33).

Our current concepts of the emphysema pathogenesis have focused on the destructive effects of protease excess in the lung (34, 35). In accord with this conceptualization, our studies demonstrate that IL-18R-dependent signaling plays an important role in regulating the levels of pulmonary proteases with CS induction of MMP-12, cathepsin-S, and cathepsin-B being mediated via IL-18Rα-dependent mechanisms. These findings are in accord with the established importance of many of these proteases in murine models of emphysema (14, 24), the known ability of IL-18 to contribute to the tissue destruction in murine models of arthritis (21, 36), diabetes (37, 38), and colitis (39) and the established ability of IL-18 to regulate MMP-2 and other MMP (40–43). They also extend our knowledge of IL-18-induced protease effector responses to MMP-12 and cathepsins S and B and demonstrate that IL-18 is an important mediator of the regulatory effects of CS on these important proteolytic moieties.

Recent studies demonstrating increased levels of structural cell apoptosis in tissues from patients with emphysema and models of this disorder (44–48) have led to the speculation that this cell death response plays an important role in the pathogenesis of emphysematous tissue destruction. Our studies demonstrate that CS induces apoptosis and activates caspases 3 and 8 in the murine lung and that signaling via IL-18Rα is required for the optimization of these responses. These observations suggest that both the extrinsic (death receptor) and intrinsic (mitochondrial) cell death pathways are being activated by IL-18. This observation is in keeping with the results of studies using a variety of extrapulmonary cells and tissues, which have highlighted the ability of IL-18 to induce apoptosis and tissue injury via intrinsic and extrinsic pathways (49–52). In addition to the cell death and mitochondrial apoptosis pathways, endoplasmic reticulum stress regulates cell viability via a pathway involving caspase-12 (53, 54).
Our studies demonstrate that caspase-12 is also activated by CS via an IL-18Rα-dependent mechanism. In so doing, these observations extend our knowledge of CS-induced and IL-18-induced apoptosis and cell death responses to include the unique endoplasmic reticulum stress, cell death pathway.

The British Hypothesis, which dominates present day thinking in airways disease, suggests that asthma and COPD are clinically, mechanistically, and pathologically distinct entities (15, 19). In accord with this traditional concept, asthma is now viewed as a chronic Th2 inflammatory disorder of the airway characterized by reversible airflow obstruction that does not develop significant lung destruction (55, 56). In contrast, the emphysema in COPD is characterized by Th1 inflammation, macrophage, and neutrophil tissue infiltration, partially reversible or irreversible airflow obstruction and alveolar destruction (7, 12, 13, 34, 35). However, in real life, these distinctions may not be as clear as initially perceived because patients are commonly encountered that manifest clinical features of asthma and COPD (15, 19), Th2 cytokines have been shown to be induced during and generate asthma-like, bronchitic and emphysematous tissue responses (16, 17, 26), and polymorphisms in Th2 cytokines have been noted to associate with COPD (57). In keeping with this complexity, the Dutch Hypothesis was proposed in 1961 (15, 19). This hypothesis suggests that, in some patients, there is clinical and mechanistic overlap between asthma and COPD. To date, however, a common abnormality or mechanism upon which these Th2- and Th1-driven responses could be based has not been defined. Our studies highlight impressive alterations in the production and activation of the IL-18/IL-18R pathway in CS-exposed mice and man. IL-18 can promote Th1 or Th2 lineage maturation, depending on underlying genetic influences and the ambient cytokine milieu (21, 23, 58). Thus, when viewed in combination, it is tempting to speculate that CS-induced alterations in the IL-18/IL-18R pathway are proximal events in the pathogenesis of pulmonary emphysema and that classic British Hypothesis manifestations and mixed asthma/COPD (Dutch Hypothesis) manifestations can occur depending on the nature of subsequent insults and underlying genetic predispositions. If true, this would provide the first mechanistic link between patients with these two related, but different, disease syndromes. It would also suggest that interventions that control the IL-18/IL-18R pathway could be effective therapies for patients with both of these patterns of disease presentation.

Previous studies from our laboratory demonstrated that IFN-γ plays an important role(s) in the pathogenesis of the pulmonary emphysema in a variety of experimental modeling systems (12–14). When viewed in combination with the well-known ability of IL-18 to regulate Th1 responses (21, 23, 55), it is tempting to speculate that IFN-γ plays an important role in the pathogenesis of the effects of IL-18 in CS-exposed mice. This hypothesis must, however, be viewed with caution because IL-18 can have IFN-γ-dependent and -independent effects and, as noted above, can contribute to Th2 as well as Th1 responses (21, 23, 55). The present studies do not formally address the relationship(s) between IFN-γ and IL-18 in CS-induced emphysema. This relationship will need to be addressed in subsequent experimentation.

To evaluate the applicability of our murine findings to human COPD, we characterized the expression of IL-18 in lung tissues from never-smokers, current smokers, and former smokers. These studies revealed elevated levels of immunoreactive IL-18 in macrophages from CS-exposed individuals. They also demonstrated that the levels of the IL-18 targets, cathepsin-S and cathepsin-B, were increased in the tissues from these patients and that these IL-18 and cathepsin findings were independent of the presence of pulmonary malignancies. Furthermore, they demonstrated that, when COPD was defined based on historical, physiologic, and chest computerized tomography scan criterion, significantly elevated levels of IL-18, cathepsin-S, and cathepsin-B were seen in tissues from patients with COPD. Interestingly, the levels of IL-18 and the cathepsins did not correlate with disease severity as assessed by GOLD staging (M.-J. Kang and J. A. Elias, unpublished observation). This conclusion, however, must be viewed with caution in light of the single point in time study design that was used and the limited number of patients in each GOLD category. Overall, these studies highlight remarkably similar responses to CS in mice and man. The demonstration that CS increases the levels of cathepsins in COPD also allows for the intriguing speculation that the novel cathepsin-S-mediated epithelial apoptosis response that has been seen in murine models of emphysema (14) is also operative in the human disorder. Additional investigations will be required to define the relationships between the CS-induced IL-18 pathway abnormalities and disease natural history and the mechanisms that underlie these relationships.

In an attempt to get a glimpse at the status of the IL-18 system in vivo, we measured the levels of IL-18 in the serum of patients with COPD. These studies demonstrated that the levels of IL-18 are higher in patients with COPD than in patients without COPD. Importantly, although diseases other than COPD have been associated with elevated levels of IL-18 (6, 21, 29, 59–64), our findings could not be attributed to these potential confounders because these diseases, when present, were similarly distributed in our patient populations. These findings raise the intriguing possibility that the levels of circulating IL-18 represent a readily accessible biomarker that reflects that activation of the IL-18 pathway in tissues. As an extension of this possibility, it would be very exciting if the IL-18 levels predicted the onset, rate of progression, or response to an IL-18 pathway-based therapeutic intervention in smokers or patients with established COPD. A biomarker of this sort would be particularly useful in COPD because only a minority of cigarette smokers actually get the disease, and the severity of the disease that they acquire varies on an individual-to-individual basis (34, 35). In accord with this possibility, the levels of circulating IL-18 are elevated in an early preclinical stage of diabetes where they foreshadow the development of the full blown disease (62). Lastly, if an IL-18 biomarker is appropriately validated it could facilitate the subsequent evaluation of IL-18-based therapeutic interventions. Specifically, the levels of circulating IL-18 could represent a rational basis on which target populations could be stratified thereby allowing therapeutic effectiveness to be evaluated in more mechanistically homogeneous patient populations.

In summary, our studies demonstrate that CS induces and activates IL-18 in mice and man and demonstrate, in a murine modeling system, that IL-18Rα plays a critical role in the pathogenesis of CS-induced inflammation and alveolar destruction. These studies suggest that interventions that block IL-18Rα may be therapeutically useful in patients with CS-induced pulmonary pathologies. Additional investigations of the roles of IL-18 and the IL-18R in the pathogenesis of COPD and other pulmonary disorders and the utility of IL-18R-based therapeutics in their treatment are warranted.

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References


